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Insights into neurogenesis and aging: potential therapy for degenerative disease?

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Abstract

Neurogenesis is the process by which new neural cells are generated from a small population of multipotent stem cells in the adult CNS. This natural generation of new cells is limited in its regenerative capabilities and also declines with age. The use of stem cells in the treatment of neurodegenerative disease may hold great potential; however, the age-related incidence of many CNS diseases coincides with reduced neurogenesis. This review concisely summarizes current knowledge related to adult neurogenesis and its alteration with aging and examines the feasibility of using stem cell and gene therapies to combat diseases of the CNS with advancing age.

Keywords

aging; cell therapy; gene therapy; neurogenesis; stem cell

There are few therapeutic options for restoring neurologic function following brain injury or disease at present. Thus, there is broad appeal for the idea that stem cells could be used as therapeutic intervention for age-related cognitive decline and neurodegenerative disorders as successfully as hematopoietic stem cells are used for the treatment of leukemia. The incidence of age-related neurodegenerative disorders can be expected to increase, given that the number of adults over the age of 65 years is expected to increase to 20% of the US population by the year 2050 [1]. As medical care continues to improve longevity, the achievement of successful aging will have tremendous individual and economic importance.

Aging, simply the act of living longer, is a major risk factor for the development of neurodegenerative disorders as illustrated by the median onset age for diseases such as Alzheimer's, Parkinson's, Huntington's, amyotrophic lateral sclerosis and others [2–4]. Although extensive research into the etiology of such diseases has yet to define a common causal factor, this inability to identify causality is congruent with the high variability of the aging process itself. If we define 'successful aging' as those older individuals who are able to function independently with intact cognitive capacity, then 'pathological aging' runs the spectrum from dementia to culturally expected forgetfulness with individuals exhibiting highly variable functional decline over a similar lifespan. Likewise, the onset and progression of neurodegenerative disorders is quite variable.

Although the existence of neurogenesis in the adult brain is now universally accepted by the scientific community, it has been a slow and often contentious process to refute the long-held dogma that no new neurons were generated in the adult brain. Additionally, elucidating details about the stages of neurogenesis and its functional significance, as well as the molecular processes guiding these events, was limited until recent technological advances allowed appropriate experimental investigation [5]. We have come incredibly far in the 40 years since adult neurogenesis was first suggested to a skeptical scientific community and it is apparent that we are ready for the next step: determining therapeutic management of this phenomenon. This review will examine the feasibility of successful use of neurogenesis through stem cell and gene therapies for the management of neurodegenerative disease and age-related cognitive decline.

Adult neurogenesis

Location of adult neurogenesis

As neurogenesis is a life-long phenomenon, it may be a variable that distinguishes ‘successful aging’ as well as being a potential therapeutic target for neurodegenerative disorders. Neurogenesis, the birth of new neurons in the CNS from a pool of endogenous stem cells, has been conclusively demonstrated in adult mammals, including humans, in two neurogenic niches; the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone of the hippocampal dentate gyrus (SGZ) [6]. Changes in neurogenesis seen with age and neurodegenerative diseases appear to vary between the SVZ and SGZ, with most studies focusing on the hippocampal SGZ owing to its greater clinical relevance. In this review, we limit discussion to the hippocampal SGZ and refer the reader to an excellent review of SVZ neurogenesis elsewhere [7].

The term ‘niche’ is borrowed from the description of the distinct microenvironment where hematopoietic stem cells reside, are maintained, and ultimately give rise to cells of the hematopoietic and stromal lineages [8]. In the brain, a neurogenic niche is defined by the presence of neural stem cells (NSCs), supporting glial cells, vascular supply and the combination of local environmental signals that maintain stem cells or initiate their asymmetric division. The neurogenic niche is described in detail in Figure 1. Understanding the neurogenic niche is an essential goal in developing any therapeutic approaches using NSCs, as the signals used within the niche are essential to guide the processes of proliferation, migration and differentiation.

The spatial restriction of these two neurogenic niches (SVZ and SGZ) is of considerable relevance to their potential role in repair of the brain. While both regions are centrally located, they represent an exceedingly small portion of the total brain and, thus, therapeutic use of stem cells from these regions will require some means to distribute the NSCs and/or their cellular progeny to distant brain regions where repair is required. Thus, while some exciting work will be reviewed below regarding the stimulated migration of neural progenitor cells out into the surrounding brain parenchyma, the clinical translation of these studies is likely to be quite limited as the distances achieved with migration in the rodent brain are insignificant when compared with the much larger size of the human brain.

Process of adult hippocampal neurogenesis

In the hippocampus, stem cells proliferate in the subgranular zone of the dentate gyrus. Within this anatomical location, a small population of NSCs (also known as Type I cells) undergo infrequent cell cycle that can result in asymmetric division producing an amplifying neural progenitor cell (ANP; also known as a neuroblast or Type II cell). In turn, ANPs can undergo rapid cell proliferation, amplifying the cell population that subsequently can undergo lineage

commitment leading to the eventual formation of mature neurons. During this process of lineage determination, neuroblasts migrate from the subgranular zone and become inserted into the mature granule cell layer. Thus, the process of neurogenesis can be defined broadly by three principle stages: proliferation, migration and differentiation. Each of these steps in neurogenesis, as well as the original maintenance and activation of NSCs, appears to be under the regulation of a number of environmental signals that initiate and/or modulate gene expression cascades that are orchestrated in a cell autonomous fashion. The process of neurogenesis has been well delineated and described in the literature [9,10].

Adult hippocampal neurogenesis produces functional neurons

Although initially vital to establish the existence of neurogenesis, it is now not enough to merely label the presence of new cells with the introduction of thymidine analogs that are incorporated into mitotically active cells. It must also be demonstrated that these cells display physiological processes that contribute to brain function. Evidence is now conclusive that these newly developed neurons in the hippocampus receive, process and convey information within the hippocampal circuitry [11]. Because new neurons in the adult brain must be able to integrate into an existing circuitry of mature neurons, differences between newly generated neurons and more mature ones could be important. It is possible that the existence of new neurons is responsible for activity-modified behavior [12]. Although it has been shown that new neurons, once mature, function in an identical manner with existing cells, recent studies investigating the activity of new and developing neurons have revealed some interesting differences. Using patch clamp recordings in acute hippocampal slices, Mongiat *et al.* demonstrated significant differences in the activation of developing neurons. Newly generated neurons appear to have a lower threshold for induction of LTP and LTD, signifying increased bi-directional synaptic plasticity [13]. Additionally, these developing cells display hyperexcitability and form synaptic connections before reaching full maturity, suggesting involvement in information processing prior to full maturation. Schmidt-Hieber *et al.* further demonstrated that adult young granule cells exhibit significantly different membrane properties than mature counterparts that appear to facilitate synaptic plasticity in the hippocampus [14]. Of particular significance, Morgenstern *et al.* demonstrated that these functional differences between newly generated and mature neurons are present in mice regardless of the animal's age, indicating that environmental changes with age may affect the available pool of stem cells or dividing progenitor cells without affecting developing neuronal function [15].

Alteration of hippocampal neurogenesis

Neurogenesis appears to be a remarkably plastic process, modifiable by numerous macro- and micro-environmental influences across the lifespan [16]. This dynamic regulation is thought to be indicative of an organism's ability to adapt to novel or changing conditions. Both stress and aging have been identified as potent downregulators of neurogenesis, raising the possibility that a dysregulation of neurogenesis may be a precipitating factor to age-related neurodegeneration [17]. In fact, neurogenesis diminishes more than 80% by advanced age in all species studied to date [18,19]. Research results conflict as to the events leading to this age-related decline and it is very probable that more than one step in the process of neurogenesis is affected by age [20–23]. Olariu, *et al.* further compared basal neurogenic levels in young adult and middle-aged male rats and discovered a smaller pool of proliferative cells that maintained the capacity to divide, migrate and differentiate [24]. Although it is possible that this diminished neurogenesis is a result of an intrinsic inability of old NSCs to respond to stimuli to proliferate, it is also plausible that diminished neurogenesis with age and with stress is due to a change in the supportive milieu or niche for proliferating stem cells and newly developing neurons [25–27]. This may be due to the age-related decline in availability of key trophic factors such as BDNF, FGF-2, IGF-1 and VEGF, which occur as early as middle age [28,29].

It is attractive to speculate that signal modification of the neurogenic process could be utilized to facilitate CNS repair. This possibility is supported by the report that restoring FGF-2 levels promotes neurogenesis in the aged dentate gyrus [30]. Other strategies investigating manipulation of the brain microenvironment have also shown promise and will be discussed in more detail later in this article.

Interestingly, the aged brain still retains the capacity to dynamically regulate this process in response to macroenvironmental changes much as does the younger brain. Thus, this experience-driven regulation of neurogenesis has been upregulated by exercise, dietary restriction and an enriched environment across the lifespan [31]. The reader is referred to an in-depth review of modification of aging and neurodegenerative disorders by diet and exercise by Mattson *et al.* [32]. These increases in neurogenesis have been correlated with behavioral improvements in cognition, even in aged and diseased rodents [33]. Further evidence seems to indicate that preventative strategies such as caloric restriction, exercise and a stimulating environment may facilitate appropriate regulation of neurogenesis into advanced age and reduce the risk of neurodegenerative disorders [31,34–37].

Neurogenesis declines with aging, at a time that coincides with increasing incidence of neurodegenerative diseases and vulnerability of the brain to injury from stroke. Understanding the effect of age on neurogenesis and determining the extent of plasticity remaining within the aging neurogenic niches will be of considerable importance, not only for the impact this has on learning and memory and olfactory discrimination, but for defining age-related deficits that can provide therapeutic candidates. The remainder of this review will consider two complementary strategies, cell-mediated and gene-delivery therapies, that are being explored for the development of therapeutic restoration of age-related impairments. Following a survey of research approaches to these strategies that are relevant to brain repair, we will identify and discuss challenges that will need to be addressed to facilitate the development of clinical therapies.

Stem cell-based therapies for brain repair

Most tissues in the body exhibit some healing or recovery following injury and disease progression. However, once a critical extent of damage has been sustained that exceeds this self-repair capacity, tissue function may become compromised and organ failure may result. Progress in understanding the biology of stem and progenitor cells has generated great interest in utilizing these cells to augment such limited recovery and has given rise to a new emphasis on regenerative medicine. Reports are now appearing demonstrating feasibility for promoting healing in various tissues as a result of grafting exogenous stem/ progenitor cells [38,39] or mobilizing stem cell niches within the body to recruit endogenous stem/progenitor cells [40–42]. The therapeutic approaches to using stem cells for brain repair are summarized in Figure 2.

The brain and spinal cord exhibit very limited recovery following injury and the complexity of cell types and circuitry render CNS function extremely vulnerable to even localized injury or disease pathology. There is little spontaneous generation of replacement cells, resulting in limited neurological recovery and sustained impairment in those afflicted. Restoring neurological function is a significant medical research priority because of the long-term disability endured by patients and the resulting loss of economic productivity and burden on the public health system for continuing care. However, a particular challenge that must be addressed in the use of stem cells therapies is to define the goal of the therapy as:

- Partial/complete reconstruction of cellular elements and their circuitry; or

- Using stem cell delivery to repair the loss of function by supporting the remaining circuitry.

Both goals are worthy of pursuit as different types of pathology may require different strategies. Nevertheless, the second goal may be achieved more readily than the first.

Stem/progenitor cells can be isolated from neurogenic regions and can subsequently be grafted back into the neurogenic environment where they have the capacity to differentiate into neurons [43,44]. While there have been very promising reports of functional integration by glial progenitor cells upon grafting into a disease model [45], grafting adult-derived neural progenitor cells into anywhere but a neurogenic region of the postnatal brain results in the absence of neuronal lineage commitment by those cells [44,46,47]. As a result, studies that have grafted stem cells into brain to evaluate therapeutic potential have either investigated the incorporation of exogenous stem cells into neurogenic regions to explore functional implications [48] or have used stem cells as cellular vectors to support dysfunctional circuitry, for example, in the treatment of Parkinson's disease [49–51]. Nevertheless, there have been some reports that generation of an injury in the target region or experimentally expressing injury-related factors can support some neuronal differentiation of neural progenitor cells grafted to nonneurogenic regions [52,53]. Such studies underscore the need to define signals absent from nonneurogenic regions that could be therapeutically expressed to sustain neuronal lineage commitment.

In contrast to the adult brain, the neonatal brain appears able to incorporate grafted exogenous stem cells with widespread distribution and survival. This capacity has been used effectively in preclinical models of demyelinating disorders [45] and lysosomal storage disease [54]. Thus, it appears that the still developing neonatal brain may retain sufficient developmental signals to support the survival, differentiation and functional integration of exogenous stem cells. This signal expression appears to be reduced with maturation with an even further diminution of such signals in the aging brain [28,55]. Nevertheless, one recent report showed that grafting stem cells to an aged neurogenic region can stimulate the local production of new neurons [56], suggesting that the aging brain may maintain the capacity for repair, at least in the neurogenic regions. More work is needed to better understand the capacity of the aged brain to both produce neurons itself and to incorporate grafted stem cells.

An alternative approach to the grafting of exogenous cells may be the recruitment of endogenous neural progenitor cells in non-neurogenic regions of the adult brain. The existence of such local endogenous progenitor cells is supported by the fact that they can be derived from a wide range of regions and grown *in vitro* under appropriate conditions to demonstrate multipotentiality [57–60]. Useful insights into how such local stem cells may be harnessed for therapeutic use comes from examining injury response models in animal studies. There is increasing evidence that the brain does mount some regenerative response to cortical injury that includes at least a transitory addition of new cortical neurons under some circumstances. Focal cortical injury stimulates proliferation within the SVZ and directs migration of newly generated neuroblasts out toward the site of injury. While some of these cells express early markers of neuronal commitment, the primary fate of these cells appears to be a glial lineage [61–64]. Ischemic injury elicits a similar proliferative and migratory response, with mobilization of neuroblasts both from the anterior SVZ to striatum and cortex [65,66] and from the posterior ventricular lining to hippocampal area CA1 [67]. It has been established that these neuroblasts are indeed arising from the SVZ and are not locally recruited progenitors [68]. Recruitment of migratory neuroblasts to the striatum or hippocampus can result in the differentiation of neurons that can survive for weeks [69]. However, apart from a recent report showing long-lasting cortical neurons in a neonatal ischemic model [70], any potential cortical contribution of new neurons appears transitory. The ischemic injury signals mobilizing proliferation and migration of neuroblasts from neurogenic niches can be augmented by the

infusion of growth factors [67,71]. Nevertheless, specific focal injury models have been shown to provide sufficient support to direct the differentiation of endogenous neural progenitor cells. While the source of these neural progenitor cells remains uncertain, their most likely source is migration from the SVZ [72,73].

Such evidence for the initiation of self-repair signals by the brain is a cause for optimism. While studies of cortical injury or ischemia document the initiation of proliferation and migration from neurogenic niches, they also note the presence of many proliferating cells near the injury that appear to be the result of local proliferation rather than migration and that typically do not show differentiation into neuronal or glial phenotypes [63,66]. Thus, the initial self-repair response of the adult brain to injury may remain incomplete due to either the lack or insufficiency of the signals that were present during development that support and direct the lineage determination of NSCs. These studies support the concept, demonstrated by the *in vitro* studies mentioned above [58–61], that despite the very restricted spatial localization of neurogenesis in the postnatal brain, a population of endogenous neural progenitor cells may exist throughout the neuraxis that could potentially be recruited for repair. Supporting this concept are studies that have succeeded in directing a neuronal fate for neural progenitor cells that originated from neurogenic zones by expression of developmental signals [74,75]. Given the insufficiency of signals for neuronal fate specification in nonneurogenic zones, particularly in the aged brain [28], the implementation of potential therapies to either direct grafted stem cells or to recruit endogenous stem cells may require the assistance of gene delivery approaches (described below) to provide the necessary signals (Figures 2B & C).

Gene therapy for brain repair

Gene therapy and the combination of gene therapy with direct stem cell manipulation have recently experienced a resurgence of optimism [76]. The classical paradigm for gene therapy is the replacement of defective genes (loss of function) with the corrected gene in critical cell populations. Clear examples of this include the treatment of X-linked severe combined immuno deficiency and adrenoleukodystrophy [77–79]. These diseases are particularly amenable to gene therapy as they can be addressed through easily accessible stem cell populations (hematopoietic) that can be transduced *ex vivo* (allowing for greater transduction efficiency) with a single gene and expanded *in vivo*. Unfortunately, in the cases of the major diseases of the CNS, there is often not a single loss of function mutation that can be replaced. Furthermore, there are multiple changes in gene expression that occur during aging that hinder neurogenesis. Therefore, multiple genes may be required to combat the disease in addition to the effects of aging. For example, gene therapy could be used to deliver secreted trophic factors to the site of injury inducing recruitment and or promoting survival. Subsequent gene delivery could be used to enhance, direct and sustain NSC populations through direct *in vivo* or *ex vivo* transduction with genes that may affect these outcomes. The synergy of these approaches is discussed further below.

The complementarity of stem cell and gene therapies is illustrated in Figure 2B & 2C. One of the major classes of signals being investigated for supporting both diseased neurons and stem cells are the trophic factors. The neurotrophins are a class of proneurogenic agents that include NGF, BDNF and neurotrophin-3 (NT3). Basic FGF (bFGF) [80] and VEGF are also important proneurogenic growth factors [81]. Vector-mediated intrastriatal growth factor overexpression alone can stimulate survival and or recruitment of SVZ neuroblasts in the adjacent striatum [82,83]. Adeno-associated virus (AAV)-mediated delivery of BDNF enhanced the recruitment of progenitor cells to the lesioned striatum and promoted neuronal differentiation [83]. Attempts at directing the cell fate of recruited NSCs has met with some success. Using gene delivery of the trophic factor BDNF, Chmielnicki *et al.* induced proliferation of neural progenitor cells in the SVZ that migrated into the adjacent striatum [84]. By coexpressing

noggin, a putative lineage instruction factor, they were able to direct neuronal differentiation within this expanded population. Following spinal cord transection, Ohori *et al.* reported that treatment with FGF2 and EGF resulted in the expansion of a population of heterogeneous neural progenitor cells within the spinal cord [85]. These neural progenitor cells could be directed to generate new neurons when they were engineered to express the transcription factor neurogenenin-2 and new oligodendrocytes when they were engineered to express the transcription factor Mash1 (using retroviral gene transfer vectors). Jiao and Chen found that, by introducing sonic hedgehog protein directly into the parietal cortex, the protein not only acted as a mitogen to cause proliferation but also resulted in a detectable population of new cells expressing immature and mature neuronal markers [86]. Based upon complementary experiments, they concluded that the expanded population was likely NG2-negative, although it is possible that some proliferating cells may have migrated from the SVZ.

Genetic manipulation of stem cells is also being developed for major neurodegenerative diseases of the CNS. The use of NGF in the treatment of Alzheimer's disease (AD) is a prime example of trophic factor gene therapy promoting survival. A Phase I clinical trial was conducted using *NGF* gene transduced autologous fibroblasts delivered to the basal forebrain. This resulted in no reported adverse events and a reduction in the rate of cognitive decline. Autopsy showed robust dendritic growth in a treated individual [87]. In another study, viral vector-mediated expression of bFGF/ BDNF in the hippocampus of an epileptic lesion model increased neurogenesis, reduced neuronal damage and ameliorated epileptogenesis [88]. In a model of stroke, *VEGF* gene transfer was found to increase neurogenesis, recruit stem cells to the infarct, and reduce infarct size [89]. In an example of *ex vivo* genetic manipulation of NSCs, Gu and colleagues generated stem cells expressing NT3 and transplanted them into 6-hydroxydopamine (6-OHDA)-lesioned Parkinsonian rats [90]. The use of NT3 more efficiently replaced tyrosine hydroxylase positive (dopaminergic) neurons and alleviated Parkinsonian deficits.

The above examples showcase the great potential of the ability to manipulate gene expression directly in NSCs or in the NSC environment. Not only can gene expression be increased through viral vector-mediated gene transfer but it can also be decreased through RNA interference (RNAi) [91]. The discovery of this evolutionarily conserved RNAi system, which uses small pieces of RNA complementary to the target gene to suppress expression, garnered the Nobel Prize in 2006 [92]. This system has been further developed so that viral vectors can facilitate the continuous expression of these RNAi molecules in cells producing long-term suppression of gene expression [93–95]. As the knowledge base improves in this field, investigators will presumably refine their knowledge of key genes and generate more efficient and specific means of stem cell therapies. The needs would vary from case to case depending on the cell type required and nature of the degeneration. In some cases it would be advantageous to generate/recruit/promote neurons and in others astrocytes or oligodendrocytes (or all of the above). Even the subtype of neuron may need to be specifically promoted (e.g., tyrosine hydroxylase, GABA). This will require detailed knowledge of key regulating factors required for recruitment and differentiation. We propose that, when possible, promoting and recruiting endogenous stem cells through genetic manipulation of the environment may represent the most plausible approach to therapeutic development, in that it utilizes the body's own unaltered stem cells in the vicinity of the injury. Furthermore, this approach does not require the genetic alteration of a stem cell population, which carries the risk of neoplastic transformation [96–98].

Ex vivo genetic manipulation may require more sophisticated means of gene transfer as permanent expression of the transgene(s) or RNAi in daughter cells may not be desirable. This would preclude the use of retroviral/lentiviral vectors (which integrate their genetic material into the host cell chromosome) in lieu of nonintegrating vectors such as AAV and adenovirus (Ad)-based vectors, which are widely used and prevalent in clinical trials [99,100].

Furthermore, *ex vivo* manipulation of NSCs is technically unrealistic as acquiring autologous NSCs from a patient is not clinically plausible. However, alternative technologies have recently been introduced for generating autologous stem cell populations that may be suitable for *ex vivo* application [101,102]. Alternatively, *in vivo* transduction and manipulation of endogenous NSCs has the advantage of reduced technical difficulty. However, targeting this stem cell population and avoiding the surrounding cells is a difficult problem. Using phage display libraries, Schmidt and colleagues were able to engineer Ad-vectors that selectively transduce NSCs *in vivo* [103]. Alternatively, integrating vectors utilizing more precise transcriptional control of transgene expression could also be used to turn gene expression on and off as desired. For example, the NSC-specific nestin gene promoter could be used to express a prodifferentiation factor and would presumably shut down as the daughter cells matured. These types of technologies will be very useful for future stem cell therapies.

Limitations to developing therapies for neurodegenerative diseases

Animal models of disease

When developing new therapeutic approaches to neurodegenerative diseases the type of model used to approximate the disease is a key component. This is particularly true when one is dealing with complex process such as aging, neuro genesis and idiopathic neurodegenerative diseases. There are two major classes of disease models. The first is genetically modified organisms carrying the mutant human gene associated with heritable forms of the disease (examples below). The majority of these models are in mice as they can be genetically manipulated relatively easily. Examples include mice carrying mutant forms of the human amyloid-precursor protein (*APP*) and presenilin (*PS*) genes associated with early-onset autosomal dominant AD (familial-AD) [104]. For Parkinson's disease (PD), an example would be mice that overexpress the mutant form of human α -synuclein associated with familial PD [105]. There are similar examples for many other diseases including Huntington's disease and prion disease. These are very useful genetic models; however, they do not necessarily replicate all the idiopathic changes that occur in clinical cases. In addition, in some cases not all the pathological features are present. For example, there is a lack of *tau* pathology in mutant *APP* transgenic (AD-like) mice unless an additional mutant *tau* gene is present [106]. It should be noted, however, that this mutant *tau* gene is not associated with AD but instead with fronto-temporal dementia. Lesion models of neurodegenerative disease are the second major class of animal model that can be used in multiple spe-used primarily in rats and mice, respectively, to kill dopaminergic neurons. The lesion approach has also been used to model AD through the use of a cholinergic basal forebrain neuron toxin (e.g., 192 IgG-saporin) [107]. These models lack the progressive age-related nature of most neurodegenerative diseases and are static in terms of pathology over the long term. Lesion models also lack the 'toxic environment' present in most age-related neurodegenerative diseases.

Obstacles to cell-mediated therapies

The therapeutic use of stem cells could be pursued through two different strategies [108]. The first strategy is the derivation, isolation and expansion *in vitro* of a suitable stem cell population. Once obtained, this population could be banked and samples used over time for therapeutic delivery (Figure 2A). Alternatively, cultured stem cell populations could be genetically modified *in vitro* to express suitable therapeutic or instructional transgenes following engraftment (Figure 2B). The second strategy would not utilize exogenous stem cells, but rather recruit local stem cells already present within the brain. As most of the brain does not support neurogenesis, a suitable environmental niche will need to be created through delivery of genes encoding for the necessary signals or factors (Figure 2C). Engineering a niche may also be a suitable strategy when paired with grafting of exogenous stem cells to areas where endogenous signals to support differentiation and functional integration are diminished or nonexistent. As

detailed below, each of these approaches faces some obstacles that will need to be overcome to facilitate clinical application.

A fundamental challenge to using exogenous stem cells is the source of those cells. Human embryonic stem cells (hESCs) have received the most attention as a source of therapeutic stem cells. As a result of their pluripotency, hESCs have the promise of the greatest flexibility for repair as they could be used for potentially any organ system. However, the process of isolating cells of the inner cell mass destroys the embryo, resulting in ethical considerations that have placed limits on this approach. More recently, it has been found that differentiated somatic cells could be induced back to a pluripotent state to essentially mimic the embryonic stem cells [109,110]. These so-called induced pluripotent cells (iPSCs) would theoretically be superior to hESCs for therapeutic delivery as they would be autologous cells and should not elicit immunogenicity. However, their therapeutic utility may be limited owing to the fact that they are genetically equivalent to the patient and any dysfunction or pathology due to genetic influences would still be present in the iPSCs. Nevertheless, iPSCs provide a valuable new tool to study neurodegenerative disease as patient somatic cells, such as skin cells, can be induced to pluripotency with subsequent differentiation into neurons *in vitro*. Access to iPSC-derived neurons from patients with specific neurodegeneration will provide unprecedented opportunities to study disease etiology and progression and to screen for therapeutic agents *in vitro*.

Another limitation of using truly pluripotent stem cells for therapeutic delivery derives from their potential to form teratomas. The probability of teratoma formation can be reduced by specifying lineage commitment prior to engraftment [111], but the presence of even a single, true pluripotent stem cell within the transplant population carries risk. As a result, considerable focus has been placed on using stem or progenitor cells with more restricted fate potential isolated from tissue. Some of these cell populations, such as the hematopoietic stem cells of the bone marrow, retain a considerable range of multipotentiality and contribute both to hematopoietic and mesenchymal stem cell lineages. Bone marrow-derived mesenchymal stem cells, as well as mesenchymal stem cells derived from other sources such as umbilical cord, are being intensely investigated for their efficacy in tissue repair [112–115]. Interestingly, the ability of mesenchymal stem cells to affect repair may derive from their ability to support an environmental niche rather than directly participate in restoration by neuronal differentiation.

The second therapeutic repair strategy described above, recruiting endogenous stem cells, has an advantage over the delivery of exogenous stem cells in that cell source and immunogenicity are no longer problems. However, the major limitation of this approach centers upon our limited understanding of the signals and factors that stimulate endogenous stem cells to proliferate and regulate their subsequent differentiation. While the identification of these signals are beginning to be elucidated for neurogenic regions of the adult brain, relatively little is known regarding environmental cues that will need to be supplied to recruit stem or progenitor cells in the majority of the brain that is non-neurogenic. There is also a need to refine knowledge of the age-related changes in environmental cues [21,28] and the effect of degenerative neuropathology [116,117] that further reduce endogenous neurogenesis so that appropriate strategies to prepare the aged and/or diseased brain to accept stem cell therapies can be developed.

Obstacles to gene therapy

Obstacles to the successful therapeutic modulation of neurogenesis by viral gene transfer include first and foremost the need for a sufficient understanding of the genes regulating key stem cell properties (e.g., proliferation, survival, specific differentiation, recruitment). Also, one may need to know precisely which therapeutic gene is required to overcome the pathology. In addition, the changes in stem cells and their niche that occur during aging must also be

understood as they could impose limitations on therapies and require approaches that compensate for age related deficiencies. Finally, an understanding of the toxic effects of the particular pathology on stem cells is critical to the development of gene therapy approaches (discussed below). Concerning technical issues related to the use of gene therapy, there are several considerations. Multiple administrations of the gene transfer vector may be necessary to maintain long-term transgene expression creating technical and immunological problems [118]. However, in the vast majority of cases this should not be an issue. This is because the nature of the currently available mainstream vectors allows for continuous transgene expression if used on long-lasting (terminally differentiated) cell populations (e.g., neurons) [119–121]. If permanent transgene expression is required from replicating stem cells then integrating retroviral vectors (e.g., lentiviral) would be required [122,123]. In this case, insertional mutagenesis and oncogenesis is a variable to consider. In some cases, the use of ‘suicide genes’ (e.g., *HSV-TK*) that can be used to kill the transduced cell in the presence of an inducer (e.g., ganciclovir) may be advantageous. Finally, immune-mediated elimination of transgenic cell populations can be avoided by using current generation viral vectors (i.e., non-immunogenic) and human transgenes (i.e., self antigens).

Obstacles to therapeutic efficacy

Neurodegenerative diseases may share many common features, but they are not monolithic and each disease will require a tailored therapeutic approach that addresses all facets of neurological impairment. The challenges associated with developing stem cell therapies for neurodegenerative diseases is well illustrated with AD. There are two facets to understanding the stem cell therapies in AD as there exists both a widespread neuronal dysfunction and/or loss to address as well as the observation that neurogenesis is reduced in AD. It appears that the reduced neurogenesis with AD can largely be accounted for by a significant decline in both the number of neuroblasts and their proliferative capacity (reviewed in [117]). Studies in transgenic mouse models of AD showed mixed effects on neurogenesis, reflecting the plethora of transgenic models and nature of the neurogenic analyses. Regardless, a majority of studies reported reduced neurogenesis (reviewed in [117]). While AD may appear amenable to stem cell therapy, there are several obstacles to overcome. First, it is primarily a disease of synaptic loss, with neural cell death as a later stage characteristic of the disease. Therefore, delivering or inducing stem cells in the brain will not directly address the most significant aspect of AD pathology. Second, there are underlying causes of this disease that produce a toxic environment leading to neuronal dysfunction and death. Therefore, replacing lost or dysfunctional neurons in this toxic environment could be compared with pumping air into a flat tire without first patching the hole. Third, the area of degeneration in AD is very large affecting the majority of the cerebral cortex and limbic system. It is difficult to imagine how so many neurons could be properly replaced. However, it should be noted that minor regional improvements in pathology (e.g., hippocampal) could still positively affect cognition and quality of life. Furthermore, these obstacles do not mean that a therapy related to neurogenic stimulation would not be successful for AD. Indeed, it may be the case that a proneurogenic environment reduces pathogenesis (toxicity) in AD (reviewed in [117,124]). Unlike AD, PD is presumably more amenable to treatment with a stem cell (cell replacement) approach. This is primarily because it is widely believed that the cause of PD is due to neuronal cell loss in a relatively small region known as the substantia nigra, pars compacta. Assuming cell replacement would result in proper reinnervation of the striatum, cell replacement seems to be a more reasonable goal for PD. However, the problem of a toxic environment that results in cell loss would presumably persist. It is unclear if neurogenesis is suppressed or stimulated in PD and the potential of NSCs to differentiate into dopaminergic neurons in the absence of genetic or pharmaceutical manipulations is also in question. A similar conclusion can be derived for amyotrophic lateral sclerosis where the effects on neurogenesis are not clear, including the potential of natural NSCs to replace motor neurons (reviewed in [124]). Perhaps the condition most amenable to

treatment using a stem cell approach is acute neuronal damage as the aforementioned caveats would not apply. This is perhaps reflected in the large number of research groups exploring stem cell approaches for more acute injury including stroke and epilepsy. Furthermore, neurogenesis is clearly upregulated in models of stroke (reviewed in [124]) suggesting that augmenting the natural response may be an appropriate therapeutic strategy.

Conclusion

A solid understanding of adult neurogenesis has emerged over the last two decades of research. We now understand that spatially restricted regions of the adult brain produce new neurons that functionally integrate into existing circuitry and progress has been made in elucidating the signals/factors that control the processes of NSC proliferation, differentiation, survival and integration. However, most of the adult brain does not support neurogenesis and additional insight is needed to understand how new neurons can integrate into these vast non-neurogenic regions. Furthermore, much remains to be discovered regarding these age-related changes in the NSC population as well as necessary environmental signals/factors to support neurogenesis in the aging brain.

At least two critical barriers to progress in developing stem cell therapies for the aging brain exist. The first of these is the identification of critical signals/factors for enhancement or suppression to assure newly generated cells achieve their appropriate differentiation and functional integration. Second, as the aging brain reduces expression of many known proneurogenic signals, it will be necessary to determine if their augmentation is adequate to restore neurogenesis and the combination of factors needed. Potential inhibitory factors for NSC proliferation and differentiation should also be identified in the event that their neutralization is an appropriate therapeutic approach. Likewise, newly generated neurons will need to be maintained for a long life of functional use to offset age-related and/or neurodegenerative impairment. The identification of such factors and the determination of their alteration in aging or neurodegenerative pathology will be needed to develop therapeutic approaches that restore these signals to sustain the therapeutic efficacy of new cells.

Finally, there is a basis for optimism that intervention in age-related degeneration by use of stem cell therapies can be developed in time to address the needs of an increasingly elderly population. Evidence exists that endogenous stem cells in the CNS retain their capacity to repopulate brain regions, even in aged individuals and, even though the capacity of the niche is reduced in the aging brain, it retains the ability to respond to proneurogenic stimulation. Furthermore, many of the technical approaches to stem cell isolation, cultivation, delivery and/or recruitment and the use of gene therapy to modify cells and engineer environments have already been developed. Thus, the good news is that much of the technology currently exists to introduce cells and engineer suitable niches within the brain, facilitating a rapid translation of preclinical knowledge to therapeutic use in the clinic.

Future perspective

There will be a pressing need for new therapies to address the age-related increase in neurological disease as we approach the estimated threshold in the year 2050 when 20% of the population will be older than 65 years of age. The combination of cell-mediated and gene therapies will likely play a central role in new therapies and the technical approaches to deliver cells and vectors to the brain are largely already in hand. Progress is largely limited by insufficient knowledge of critical regulatory signals and the way in which these act in concert to guide cell fate. It is likely that progress will be incremental as we acquire adequate knowledge concerning the necessary spatial and temporal concentration at which regulatory signals should be presented, either individually or in combination, to promote repair. However, once this

degree of understanding is achieved, the development of successful therapies may proceed rapidly.

Executive summary

Adult neurogenesis

- This is a lifelong phenomenon occurring in two neurogenic niches.
- New neurons make a functional contribution.
- Regulation of neurogenesis is experience-driven and readily modifiable.
- Aging brain retains the capacity to generate new neurons in response to appropriate stimulation.

Stem cell-based therapies for brain repair

- The goal of stem cell therapies can be defined as:
 - Partial or complete reconstruction of cellular elements and their circuitry.
 - Repair by supporting function of remaining circuitry.
- In the neonatal brain, grafted stem cells are widely incorporated.
- In the adult brain, stem cells must be grafted to a neurogenic region to differentiate.
- Relatively little is understood regarding the fate of cells grafted to aging brain.
- An alternative strategy to grafting exogenous stem cells is to recruit rare, endogenous stem cells in non-neurogenic areas.

Gene therapy for brain repair

- Stem cells can be genetically modified prior to engraftment.
- *In vivo* gene delivery can be used to engineer a niche to support grafted cells.
- Similarly, a niche can be engineered to recruit endogenous stem cells.
- Gene delivery can elevate expression or suppress expression through RNA interference.
- Different viral vectors and promoter combinations offer specific advantages and disadvantages.

Limitations to developing therapies

- Animal models of disease often do not faithfully recapitulate all disease pathology.
- Sourcing exogenous stem cells may present challenges and produce variability.
- Recruiting endogenous stem cells is limited by poor knowledge of regulatory signals.
- Widespread degenerative diseases with progressive dysfunction that precedes cell loss represent the greatest challenge for stem cell therapies.
- The effects of neurodegenerative pathogenesis on neurogenesis need to be better understood.

Conclusion

- Neurogenesis persists in the aged brain, although at a reduced level.
- Neurogenesis in the aged brain can be enhanced.

- Techniques to introduce cells and engineer niches within the brain are available.
- Critical barriers to progress in stem cell therapies for the aging brain are:
 - Identification of signals/factors that protect stem cells and guide their appropriate differentiation and functional integration.
 - Identification of signals/factors that condition the environment to maintain the long-term therapeutic efficacy of new cells.
- Translation to therapeutic utility requires increased knowledge of regulatory signals/factors regulating neurogenesis, particularly in the aging brain.

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Figure 1. Neurogenesis in the adult brain is maintained in a specialized microenvironment; the neurogenic niche

(A) Neurogenesis, the production of new neurons, is spatially restricted in the mature brain to two regions, including the hippocampus within the medial temporal lobe of the human brain (boxed region). (B) Within the hippocampal formation, neurogenesis is further spatially restricted to the dentate gyrus (boxed region). (C) The dentate gyrus contains a small population of cells, clustered along the subgranular zone, that extend long apical process through the granule cell layer with terminal branching. This population of neural stem cells, designated as Type I cells, cycle slowly, express markers of immature cell phenotype, and produce, through asymmetric division, progeny, called amplifying neural progenitor cells (also known as Type

II cells), that rapidly proliferate and begin to differentiate. Those neuroblasts that survive and successfully complete neuronal cell lineage specification become mature neurons that functionally integrate into the granule cell layer. The process of cell proliferation and differentiation is regulated by the local environmental expression of stimulatory and/or inhibitory signals (paired green/red arrows) that can be derived from local glial cell populations or from the local vasculature. The expression of suitable signals at an appropriate concentration orchestrates the process of stem cell proliferation, survival and differentiation. Elucidating the role of these signals will be crucial for developing therapeutic engineering of an environmental niche to stimulate endogenous neurogenesis, support the differentiation of grafted stem cells, or initiate the recruitment of endogenous stem cells in the brain for repair.

Figure 2. Approaches to the therapeutic use of stem cells

(A) Exogenous stem cells may be isolated and expanded *in vitro* in preparation for grafting into the brain by stereotaxic injection. The exogenous stem cells could come from a variety of sources including embryonic stem cells or tissue stem cells. Tissue stem cells could be isolated from a variety of somatic tissues, from bone marrow, or from extra-embryonic tissue, such as the umbilical cord. Induced pluripotent stem cells, derived from somatic cells that have been reprogrammed to pluripotency, may be used in research studies for the benefit they offer for understanding disease pathology. Thus, by introducing diseased stem cells to healthy brain or healthy stem cells to diseased brain, induced pluripotent stem cells could be useful for discriminating the cell autonomous deficiencies of diseased neurons relative to the environmental support offered by the diseased brain. (B) Cultured stem cells could also be genetically modified *ex vivo* prior to stereotaxic injection into the brain. This approach could be useful for expressing cell autonomous signals within grafted stem cells that, through autocrine or paracrine interactions, would promote successful neuronal differentiation. Alternatively, the *ex vivo* genetic approach could be useful for modifying stem cells to express a therapeutic transgene, thus using the stem cell as delivery vectors. (C) Gene delivery could

occur directly into the CNS by stereotaxic injection to encode infected cells to express signals/factors necessary to sustain a neurogenic niche for grafted stem cells. Alternatively, *in vivo* gene delivery could achieve the expression of appropriate transgenes for the local recruitment of endogenous stem cells. Such therapeutic niche engineering could be used for reversing age-related decline within neurogenic niches or generating a neurogenic niche in regions that otherwise did not express the necessary signals/factors. **(D)** If suitable signals/factors supporting neural stem cells or recruiting endogenous neural stem cells can be identified, it may prove possible to move beyond cell- or gene delivery-mediated therapies and systemically deliver drugs or small molecules to the brain instead.